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短　報

Examination of wood sampling method with an increment borer: An investigation of seasonal changes in vessel formation

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成長錐を用いた木片試料採取法の検討
—道管形成の季節変化を調べる観点から—

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Key words: increment borer, sampling method, vessel formation, ring-porous species, diffuse-porous species

キーワード：成長錐，試料採取方法，道管形成，環孔材樹種，散孔材樹種

1. Introduction

Relationships between vessel formation and leaf phenology in trees are important aspects of their adaptive strategies. For example, the timing of vessel formation and leaf emergence differs between ring-porous and diffuse-porous trees growing in the same area (Wareing 1950, 1951; Ladefoged 1952; Suzuki *et al.* 1996, 2000; Frankenstein *et al.* 2005), suggesting that functional differences in water conducting regimes have a close relationship to leaf phenology. In research of this kind, however, the sampling is always problematic because frequent examination to trace the periodic process of vessel formation may cause considerable damage to the trees studied.

In anatomical studies, two methods, periodical sampling of wood blocks (cambial block method) and cambial marking, have been used. In the cambial marking method, a small wound is inflicted periodically on the cambium with a pin, knife, or other tools; wood blocks including marking positions are then collected. By this method, the radial increment of the xylem between the marked periods is recognized. This technique was originally developed by Wolter (1968) and investigated extensively by Kuroda *et al.* (1984a, b, 1985). It has been used to measure radial increments of tropical trees (Shiokura 1989; Nobuchi *et al.* 1995; Callado *et al.* 2001; Mukogawa *et al.* 2003) and temperate trees (Nobuchi *et al.* 1993). Because the markings cause only small wounds, more marks can be made within a small area of a stem. However, there is a time lag between the date of marking and the time for the tissue around the mark to form completely. Consequently, the analysis of anatomical features becomes more complicated.

In the cambial block method, samples are collected by cutting off small cubic wood blocks containing bark, phloem, cambium, and xylem using a chisel or knife. This method has been used to measure the growth dynamics of tropical trees (Fujii *et al.* 1999) and to trace cambial activity and vessel formation (Wareing 1951; Ladefoged 1952; Imagawa *et al.* 1970, 1972a, b; Wakuta *et al.* 1973; Suzuki *et al.* 1996, 2000; Farrar *et al.* 1997). It has the advantage that cambial activity can be observed, although it takes more time and effort for sampling and processing, and sample trees receive larger wounds.

For periodical and frequent sampling of a tree, a less destructive and more convenient sampling method is necessary. In this study, we used an increment borer to collect wood core samples to reduce damage and sampling effort. The increment borer is a common tool in tree ring research. The method has been used for analyses of dendrochronology (Carrer *et al.* 2007), stem

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growth (Nishizawa 1955; Toda *et al.* 1995), histochemistry (Nobuchi *et al.* 1976, 1979, 1986), and seasonal changes in vessel formation (Ladefoged 1952; Fonti *et al.* 2007). Increment borers are not usually used in analyses of cambial activity, because the cambial zone is often destroyed. However, for the analysis of the time course of vessel formation from the viewpoint of water conduction, sampling with an increment borer is considered useful. Ladefoged (1952) used increment boring to trace seasonal changes in vessel formation, but did not provide a detailed description.

In this paper, we describe the increment borer method in detail. The degree of tree wounding, simplicity of collecting wood core samples, and quality of wood core samples obtained with an increment borer were investigated.

2. Materials and Methods

2. 1. Sampling

Katsura (*Cercidiphyllum japonicum*: diameter at breast height [DBH] = 19-65 cm, tree height [H] = 9-28 m) and keyaki (*Zelkova serrata*: DBH = 21-30 cm, H = 13-15 m) were used as diffuse- and ring-porous wood species, respectively. Five trees of each species were selected at the Ashiu Forest Research Station (30° 18′ N, 135° 45′ E, 600-700 m above sea level) of the Field Science Education and Research Center, Kyoto University, Japan. The descriptions of sample trees are listed in Table 1. Cylindrical wood core samples (7 mm in diameter, 20 mm in length) were collected with an increment borer (Matson; Figure 1) near breast height (1.3 ± ca. 0.2 m above ground) facing south whenever possible, but from the upper side of slope when trees were growing on a steep slope, and fixed with 3% aqueous glutaraldehyde. The holes caused by sampling were about 8 mm in diameter and about 25 mm in depth (Figure 2). The horizontal distance between holes was set at least 20 mm apart. This distance is sufficient to avoid the influence of previous wounding, based on a previous study (Okada *et al.* 1993) in which the discoloration caused by wounding was 5 mm in the tangential direction 4 months after boring. No visual influence of the wound response after sampling, such as abnormal tissue formation, was observed. Sample collection was carried out biweekly (on 22 or 23 April, 6 or 7 and 22 May, 3 and 17 June 2004). All holes were plugged with silicon resin immediately after each sampling.

2. 2. Microscopic observation of vessel formation

Wood core samples were cut into 5-10 mm long segments in the radial direction. Transverse sections (15-

Table 1. Description of sample trees and the dates of vessel formation.

Species	Porosity	Tree number	DBH*1 (cm)	H*2 (m)	The dates of vessel formation: 22 or 23 Apr	6 or 7 May	22 May	3 June	17 June
Katsura	Diffuse-porous	Cj-242	19	9	a*3	b*3	c*3		
(*Cercidiphyllum*		Cj-243	32	18	a	a	b	c	
japonicum)		Cj-244	65	28	a	a	a	b	c
		Cj-245	62	27	a	a	b	c	
		Cj-246	42	24	a	a	a	a	a
Keyaki	Ring-porous	Zs-202	30	15	b*4	c*4		d*4	e*4
(*Zelkova*		Zs-203	22	13	a*4	c		d	e
serrata)		Zs-204	21	14	b	c		d	e
		Zs-205	30	13	a	b	c,d	e	
		Zs-206	24	14	b	c		d	e

*1DBH: Diameter at breast height *2H: Tree height *3Vessel formation of katsura. a; No vessels are evident. b; The first-formed vessels adjacent to the annual ring border (the first-formed vessels) are enlarged. c; All first-formed vessels take shape with the progression of their lignification. *4Vessel formation of keyaki. a; The first-formed wide vessels adjacent to the annual ring border (the first-formed wide vessels) are enlarged. b; Some first-formed wide vessels are lignified and others are not. c; All first-formed wide vessels take shape with the progression of their lignification. d; The first-formed narrow vessels in the non-pore zone are enlarged but not yet lignified. e; All first-formed narrow vessels in the non-pore zone are lignified.

30 μ m in thickness) were cut using a sliding microtome equipped with a freezing unit, double-stained with 1% safranine (in 50% ethanol, about 20 min) and 1% fast green (in 95% ethanol, about 1 min), dehydrated with an ethanol series (50, 70, 80, 90, 95, and 100%), ethanol replaced with xylene, and then mounted in Canada balsam on glass slides for light microscopy (Sass 1951). Staining with safranine served as an approximate method to judge the lignification of the vessels. In addition, the result of double staining with safranine and fast green was compared to that of the phloroglucinol-hydrochloric acid reaction.

3. Results and Discussion

3. 1. Observation of the time course of vessel formation

In katsura (Cj-243), a diffuse-porous species, formation of vessels was not observed on 23 April (Figure 3a); enlargement of the first-formed vessels was observed next to the annual ring border on 22 May (the first-formed vessels; Figure 3b). All first-formed vessels developed with progression of their lignification on 3 June (Figure 3c). From these observations, it was evident that lignification of all first-formed vessels started after 22 May but before 3 June. The process of vessel formation in Cj-242, -244 and -245, but not -246, was basically the same as that in Cj-243 (Figure 3, Table 1). All first-formed vessels started secondary cell wall lignification between 22 May and 17 June in katsura (Table 1). In Cj-246, no vessels were formed in the stem between 23 April and 17 June (Table 1), although this tree flushed many new leaves. The reason for this is not clear; however, we consider that this tree might have transported enough water to evaporate from the leaves without the need for new vessels, or that some new vessels might have been formed in the stem facing directions in which wood core samples were not collected. This tree had a large diameter at breast height and it is known that water is transported through vessels of several outer growth rings in the stem of diffuse-porous trees (Kozlowski *et al.* 1963), such as katsura.

In keyaki (Zs-203), a ring-porous species, the enlargement of the first-formed wide vessels was initially observed on 22 April (Figure 4a), while in another tree (Zs-204), lignification of some first-formed wide vessels was observed, but not of others, on 22 April (Figure 4b, Zs-204); all first-formed wide vessels took shape with progression of their lignification on 6 May (Figure 4c). Enlargement of the first-formed narrow vessels in the non-pore zone was observed, but without lignification, on 3 June (Figure 4d). Then, lignification of all first-formed narrow vessels in the non-pore zone was observed on 17 June (Figure 4e). From these observations, it was evident that lignification of all first-formed wide vessels started after 22 April but before 6 May, while lignification of narrow vessels in the non-pore zone began after 3 June but before 17 June. The process of vessel formation in Zs-202, -204, -205, and -206 was basically the same as that in Zs-203 (Figure 4, Table 1), although the first-formed wide vessels were all unlignified in some trees and occasionally unlignified in the others two weeks before all first-formed wide vessels took shape with progression of their lignification, and unlignified first-formed narrow vessels in the non-pore zone were observed when all first-formed wide vessels were lignified in Zs-205. All first-formed wide vessels started secondary cell wall lignification between 6 May and 22 May in keyaki (Table 1).

Comparing the development of the first-formed vessels between katsura and keyaki, katsura, a diffuse-porous species, tends to produce the first-formed vessels later and with more variation in timing of development among individuals than does keyaki, which is ring-porous. This suggests that the difference in timing and variation in vessel formation between the two species is related to differences in their life strategies.

Therefore, vessel formation of the stems in diffuse- and ring-porous trees could be traced using samples collected with an increment borer. The increment borer method was later applied to five diffuse-porous and five ring-porous species, the results of which will be described in a separate report.

3. 2. Quality of judging of vessel lignification

The result of double staining with safranine and fast green was compared to that of the phloroglucinol-hydrochloric acid reaction. In practice, the same results were found for the two methods when they were applied to transverse sections of the same sample. For example, when the first-formed vessels were not stained with safranine (katsura Figure 3b, keyaki Figure 4a), they were also not stained with phloroglucinol-hydrochloric acid (katsura Figure 5a, keyaki Figure 5c), and when they were stained with safranine (katsura Figure 3c, keyaki Figure 4c), they were also stained with phloroglucinol-hydrochloric acid (katsura Figure 5b, keyaki Figure 5d). Based on a comparison of the results, the double-staining method is considered to be applicable to investigations of vessel formation, including the timing of lignification.

3. 3. Examination of sample quality

The outermost part of a core sample including the bark and cambial zone was often detached from the sample during extraction of the wood core samples from the increment borer, or while cutting transverse sections using a sliding microtome, especially during the growing season, although sometimes it was not detached (Figures 3b, c). Therefore, it is usually not suitable to observe periodic changes in cambial activity. Thus, the increment borer method has not been used to trace wood formation. In this study, our main purpose was to determine the timing of vessel formation, especially its lignification. For this reason, wood core samples collected with an increment borer even without a cambial zone can be used.

Our observation of transverse sections revealed that naturally lignified vessels, and even the unlignified vessels, remained in the outermost part of the section, as shown in Figures 3b and 4a, b, d (gray arrow). This result concurred with the observations reported by Priestley *et al.* (1933). In addition, transverse sections cut from the samples of the bark and cambial zone detached from the wood showed no vessels in this side (Figure 6). However, care should be taken in judging whether a vessel element can conduct water based on the safranine-stained secondary wall, because vessel elements with thick secondary walls sometimes retain cytoplasm. Observation of the radial section is helpful when it is uncertain whether a vessel element functions.

The negative effect of taking samples with an increment borer is small, and sample trees were still viable 2 years after boring. Therefore, vessel formation could be traced in such viable sample trees.

In the future, new methods must be developed to reveal the adaptive strategies of trees and to accurately trace the periodic process of vessel formation less destructively but more conveniently.

Acknowledgments

We thank the staff of Ashiu Forest Research Station of the Field Science Education and Research Center, Kyoto University, for providing sample trees, the Laboratory of Tree Cell Biology, Graduate School of Agriculture, Kyoto University, for allowing us to use a freezing microtome and for helping us to examine the phloroglucinol-hydrochloric acid reaction, and the Laboratory of Forest Utilization, Graduate School of Agriculture, Kyoto University, for helping us with the sample collection. We also thank Dr. Akira Osawa for critical reading of the manuscript and for helpful suggestions.

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(2008年9月6日受理)

Figure 1. Collecting a wood core sample with an increment borer near breast height.

Figure 2. Sampling hole (diameter: ~8 mm, depth: ~25 mm).

Figure 3. Transverse sections of wood around the outer annual ring in a diffuse-porous tree, katsura (*Cercidiphyllum japonicum*). (a) No vessels are evident (Cj-243, 23 April 2004). (b) The vessels first formed next to the annual ring border (the first-formed vessels) are enlarged, but not yet lignified (gray arrow; Cj-243, 22 May 2004). (c) All first-formed vessels are lignified (black arrow; Cj-243, 3 June 2004).

Figure 4. Transverse sections of wood around the outer annual ring in a ring-porous tree, keyaki (*Zelkova serrata*). (a) The wide vessels first formed next to the annual ring border (the first-formed wide vessels) are enlarged, but not yet lignified (gray arrow; Zs-203, 22 April 2004). (b) Some first-formed wide vessels are lignified (black arrow) and others are not (gray arrow; Zs-204, 22 April 2004). (c) All first-formed wide vessels are lignified (black arrow; Zs-203, 6 May 2004). (d) The first-formed narrow vessels in the non-pore zone are enlarged, but not yet lignified (gray arrow; Zs-203, 3 June 2004). (e) All first-formed narrow vessels in the non-pore zone are lignified (black arrow; Zs-203, 17 June 2004).

Figure 5. Transverse sections of wood around the outer annual ring with the phloroglucinol-hydrochloric acid reaction in a diffuse-porous tree, katsura (*Cercidiphyllum japonicum*) and a ring-porous tree, keyaki (*Zelkova serrata*). (a) The vessels first formed next to the annual ring border (the first-formed vessels) are enlarged, but not yet lignified (gray arrow; Cj-243, 22 May 2004). (b) All first-formed vessels are lignified (black arrow; Cj-243, 3 June 2004). (c) The first-formed wide vessels are enlarged, but not yet lignified (gray arrow; Zs-203, 22 April 2004). (d) All first-formed wide vessels are lignified (black arrow; Zs-203, 6 May 2004).

Figure 6. Double-stained transverse section containing phloem and cambium (arrow; cambium, arrowhead; sieve tube) (Cj-244, 7 May 2004).

Figure 1

Figure 2

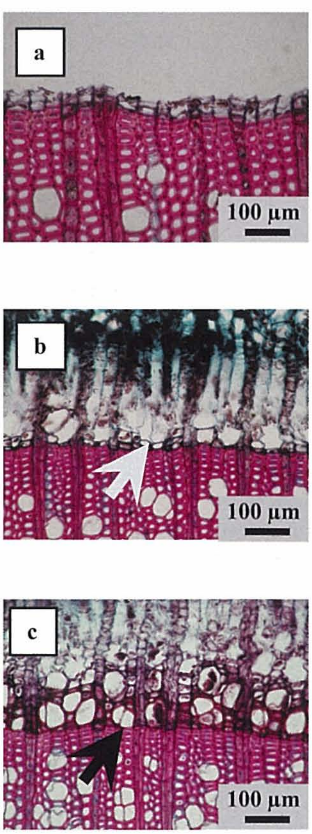

Figure 3

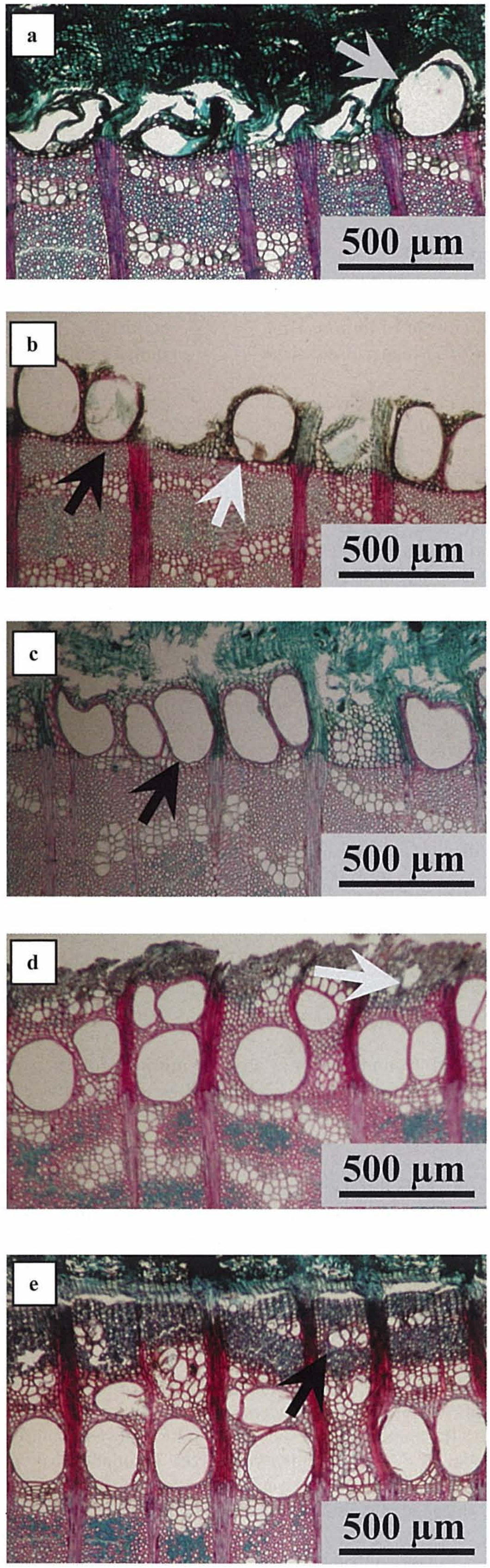

Figure 4

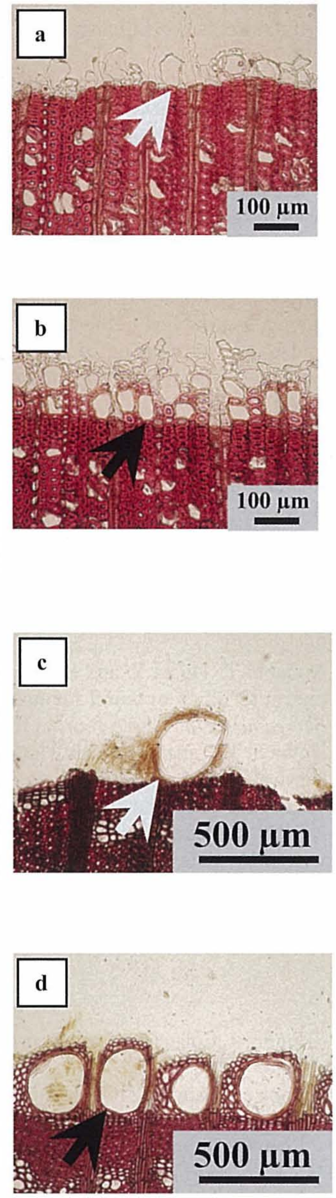

Figure 5

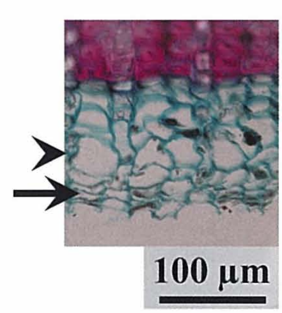

Figure 6